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TITLE: EF5 PET of Tumor Hypoxia: A Predictive Imaging Biomarker of Response to Stereotactic Ablative Radiotherapy (SABR) for Early Lung Cancer

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14. ABSTRACT

Purpose and scope:

Stereotactic ablative radiotherapy (SABR) has become a new standard of care for early stage lung cancer in patients who are not candidates for surgery because of excessive surgical risk, and will be an important treatment option for a growing segment of patients with lung cancer. This is particularly true as lung cancer screening efforts are expected to diagnose a greater proportion of lung cancers at earlier stages, yet the aging of the population will lead to a greater proportion of patients having comorbidities that increase surgical risk. Tumor hypoxia is a major known mechanism of radiation resistance and is especially expected to affect very short courses of radiation as in SABR. Imaging using a third generation hypoxia PET agent, ^{18}F-EF5, is a promising approach for noninvasive hypoxia measurement that needs to be validated in the clinical setting.

Our objectives are (1) to understand the prevalence of hypoxia detectable by imaging in early stage NSCLC; (2) to validate ^{18}F-EF5 PET as an indicator of tumor oxygenation status in this patient population; and (3) to evaluate ^{18}F-EF5 PET as a prognostic imaging biomarker for local primary tumor control after SABR.

Progress, results, and major findings:

All institutional and DOD human subjects approvals are complete and current. The first patient has been enrolled and has successfully completed all study procedures with good technical quality, and is currently up-to-date in follow up. In order to inform the remainder of the current study, we conducted a preliminary analysis including four additional previous patients from a parallel study in our department who meet the eligibility requirements of the current study.

With respect to the primary endpoint, 4 of 5 patients had imageable hypoxia as defined by our plan of analysis. With respect to the endpoint of imaging response to tumor oxygenation perturbation, 2 of 4 patients with initially positive EF5 scans had the expected imaging response to carbogen. One patient with an initially negative EF5 scan did not have the expected imaging response to DCA. Follow up is ongoing for the endpoint of local primary tumor control, but is too short to assess this endpoint meaningfully.

An action plan is in place to increase patient accrual according to the originally planned schedule.

Significance:

In summary, our preliminary findings suggest the presence of tumor hypoxia even in relatively small, early stage lung cancer. Additional validation of this finding is pending completion and analysis of our study. Because tumor hypoxia is a strong mechanism of radioresistance, particularly for hypofractionated courses of radiation as in SABR, if validated this finding has substantial implications for the optimal application of SABR to early stage lung cancer. EF5-PET imaging could be useful as a risk stratification factor for clinical trials of lung cancer SABR, and could ultimately be used to individualize therapy for patients with early stage lung cancer.

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INTRODUCTION:

Stereotactic ablative radiotherapy (SABR) has become a new standard of care for early stage lung cancer in patients who are not candidates for surgery because of excessive surgical risk, and will be an important treatment option for a growing segment of patients with lung cancer. This is particularly true as lung cancer screening efforts are expected to diagnose a greater proportion of lung cancers at earlier stages, yet the aging of the population will lead to a greater proportion of patients having comorbidities that increase surgical risk. Tumor hypoxia is a major known mechanism of radiation resistance and is especially expected to affect very short courses of radiation as in SABR. Imaging using a third generation hypoxia PET agent, ^{18}F-EF5, is a promising approach for noninvasive hypoxia measurement that needs to be validated in the clinical setting. Our objectives are (1) to understand the prevalence of hypoxia detectable by imaging in early stage NSCLC; (2) to validate ^{18}F-EF5 PET as an indicator of tumor oxygenation status in this patient population; and (3) to evaluate ^{18}F-EF5 PET as a prognostic imaging biomarker for local primary tumor control after SABR. If accomplished, these would lay the foundation for future prospective therapeutic clinical trials using ^{18}F-EF5 PET as a stratification factor, and ultimately to individualize therapy.

BODY:

Initiation of this project was initially delayed in response to revisions requested by the DOD human subjects review process. We received the approval memorandum from DOD to start the project on February 13, 2013. As such, the period covered by this report is February 13 to August 14, 2013. The tasks in the Statement of Work pertaining to project months 1-6 and the progress on each are described below.

A. Progress on Statement of Work by task (only tasks for months 1-6 included)

Task 0. ***Pre-award preparation (4 months prior to award)***

0a. Application for Stanford institutional review board (IRB) and scientific review committee (SRC) approval (4 months)

0b. Recruitment of clinical research coordinator (2 months prior to award)

Status: *Completed during pre-award period*.

All institutional and DOD human subjects and SRC approvals are complete and current. A clinical research coordinator is in place.

Task 1. ***Patient recruitment (months 1-24)***

1a. Recruitment of first 10 patients (months 1-6). Eligibility screening and recruitment will take place in the Radiation Oncology clinic as well as the Multidisciplinary Thoracic Tumor Board at Stanford Cancer Institute. After recruitment of the first 10 patients or month 6, whichever is first, we will evaluate the accrual rate and logistics to determine if any changes are needed to our procedures and workflow.

1b-1c (scheduled for project months 7-24)

Status: *Behind schedule*.

Since February 13, 2013, one patient has been enrolled on and has completed the study procedures, and is currently in follow up. An additional seven patients were screened for eligibility and met criteria. One patient agreed to participate, but prior to enrollment was found to have metastatic disease that warranted a change in therapeutic strategy (systemic chemotherapy), and was thus no longer eligible to participate. The other six patients declined to participate for logistical reasons (long distance to travel or inconvenience to come for the extra scans). As such, we are currently below our target accrual of 10 patients in the first 6 months. Strategies to mitigate this barrier to enrollment are discussed below in the Conclusions.

Task 2. Patient follow-up (months 4-34)

2a. Completion of case report forms at each follow-up visit (months 4-34)

2b. Review of data from first 5 patients (months 3-5). The study team will assess any technical barriers to collecting all the required imaging data for the first 5 patients, and address deficiencies if necessary.

2c. Semi-annual internal data review (every 6 months). The study team will internally audit all data collected on the study to ensure complete collection of study endpoints including imaging data. Missing information will be reconciled.

Status: *Complete for patient enrolled to date*.

Follow up is complete to date for the 1 patient enrolled. There were no technical barriers to completing the EF5-PET imaging studies. Data collection is complete to date. Of note, in our department we have been conducting two other clinical trials incorporating EF5-PET imaging. Early in our experience with EF5-PET, a technical challenge was the reliability of radiolabeled EF5 synthesis. These issues have been resolved through our experience with these other clinical trials, and our production of radiolabeled E5 has been very consistent with high yields. As such, we anticipate no technical barriers for this study.

Task 3. Data analysis (months 4-36)

3a. Preliminary analysis of ^{18}F-EF5 PET imaging data from first 5 patients (months 3-5). We will evaluate the image quality and technical adequacy to perform all the quantitative analysis specified by the protocol. We will also assess whether modifications to the software are needed to streamline and automate data analysis, and implement the improvements.

3b-3g (scheduled for project months 13-36)

Status: *Complete for patient enrolled to date*.

We have preliminarily analyzed the EF5-PET imaging data for the 1 patient enrolled. The results of this analysis are described below in sub-section B. The image quality was high. An important contributing factor was the good synthetic yield of radiolabeled EF5 for the imaging doses, as well as the use of a newer scanner recently acquired by our department, which has a higher imaging sensitivity and resolution.

B. Preliminary analysis of data to date

The primary endpoint of this study is to estimate the proportion of patients with early stage non-small cell lung cancer (NSCLC) undergoing stereotactic ablative radiotherapy (SABR) who have imageable hypoxia, *ie*, tumor uptake of EF5 on PET imaging that is significantly above the background uptake in muscle based on the statistical criteria defined in the plan of analysis in the proposal. The secondary endpoint is to determine if interventions intended to perturb tumor oxygenation status are reflected in the tumor uptake of EF5 on a repeat scan: patients with a positive initial EF5 scan undergo carbogen breathing (expected to decrease hypoxia) prior to a second EF5 scan, and those with a negative initial EF5 scan receive a single dose of dichloroacetate (DCA) (expected to transiently increase hypoxia) prior to a second EF5 scan. An additional secondary endpoint is to correlate imageable hypoxia with local primary tumor control after SABR.

The initial results for the enrolled patient are provided in Table 1.

Histology	Stage – Location	TMR1	Hypoxic	Intervention	TMR2	Hypoxic	Change	Expected
Adeno	IA – LLL	1.19	Yes	Carbogen	1.31	Yes	↑	↓

Table 1. Abbreviations: Adeno = adenocarcinoma; LLL = left lower lobe; TMR = tumor:muscle EF5 uptake ratio; Yes = TMR significantly > 1

At this point, no statistical analysis is possible with a single patient. Follow up for the secondary endpoint of local primary tumor control continues.

Of note, we have been conducting a parallel study of EF5-PET imaging with a very similar design, but for a much broader population of patients with different tumor primary sites, histologies, and stages. Prior to the initiation of the current project, four patients enrolled on our parallel study met the inclusion criteria of the current study and had the same study procedures (*ie*, early stage NSCLC treated with SABR, with two pre-treatment EF5 scans and tumor oxygenation perturbation intervention as per the current study). As such, a preliminary analysis including these patients is presented here in Table 2 as it is relevant to the current study.

Histology	Stage – Location	TMR1	Hypoxic	Intervention	TMR2	Hypoxic	Change	Expected
Adeno	IA – LLL	1.19	Yes	Carbogen	1.31	Yes	↑	↓
Adeno	IA – LUL	1.53	Yes	Carbogen	1.07	Yes	↓	↓
SCC	IB – RLL	0.66	No	DCA	0.54	No	↓	↑
Adeno	IA – RLL	1.16	Yes	Carbogen	1.05	Yes	↓	↓
Adeno	IB – RML	1.45	Yes	Carbogen	1.66	Yes	↑	↓

Table 2. Abbreviations: Adeno = adenocarcinoma; SCC = squamous cell carcinoma; LLL = left lower lobe; LUL = left upper lobe; RLL = right lower lobe; RML = right middle lobe; TMR = tumor:muscle EF5 uptake ratio; Yes = TMR significantly > 1; DCA =

dichloroacetate; Blue = data from previous patients in parallel study meeting eligibility criteria for current study

Preliminarily, with respect to the primary endpoint, 4 of 5 patients had imageable hypoxia as defined by our plan of analysis. With respect to the endpoint of imaging response to tumor oxygenation perturbation, 2 of 4 patients with initially positive EF5 scans had the expected imaging response to carbogen. One patient with an initially negative EF5 scan did not have the expected imaging response to DCA. Follow up is ongoing for the endpoint of local primary tumor control, but is too short to assess this endpoint meaningfully.

KEY RESEARCH ACCOMPLISHMENTS:

- All human subjects approvals obtained
- First patient enrolled and completed all study procedures; follow up current to date
- Scans of good technical quality
- Seven additional eligible patients screened
- Preliminarily, analysis of similar patients from a parallel study in our department corroborate the findings in the first patient on this study

REPORTABLE OUTCOMES:

None to date.

Publication of results (Task 4 of Statement of Work) is scheduled for project months 30-36.

CONCLUSIONS: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

The preliminary analysis to date supports the hypothesis that a substantial proportion of patients with early stage non-small cell lung cancer have tumor hypoxia that can be detected by EF5-PET imaging. A more detailed image analysis is ongoing to optimize the quantitation of tumor EF5 uptake. This includes evaluating the stability of uptake in the background regions between the serial scans and whether the background in muscle or the mediastinal blood pool may be a more appropriate reference; evaluating the sensitivity of region of interest delineation to lesion size and partial volume effects and if so, approaches for mitigating these effects.

With respect to the endpoint of being able to detect a change in tumor oxygenation status after interventions expected to perturb tumor oxygenation, additional patients will need to be studied to assess whether there is a statistically significant effect. Similarly, longer clinical follow up will be required to assess correlation of EF5 uptake with local primary tumor control.

Based on the number of eligible patients screened, we anticipate that our patient volume will be sufficient to complete this study. However, clearly a substantial barrier to patient enrollment is the logistical challenge of coming for two extra appointments to receive the two EF5-PET scans. Particularly because SABR offers a much shorter treatment course than conventional radiation therapy, similar to surgery that involves a short course of treatment, patients are coming for treatment from a large geographic region. Extra trips present a significant burden to these patients.

Action plan: To address this issue, we will modify our workflow to combine the EF5-PET scan dates with existing appointments, such as pre-treatment appointments for radiation therapy simulation or pulmonary function testing. We anticipate that this should be possible for the majority of patients and would eliminate this consistent barrier to enrollment. This would require no modification to the clinical trial protocol. Since we have encountered no other barriers, and have been able to complete all scans with good technical quality and have had complete patient follow-up to date, we expect this action plan to help bring us back into alignment with the originally planned study timeline.

In summary, our preliminary findings suggest the presence of tumor hypoxia even in relatively small, early stage lung cancer. Additional validation of this finding is pending completion and analysis of our study. Because tumor hypoxia is a strong mechanism of radioresistance, particularly for hypofractionated courses of radiation as in SABR, if validated this finding has substantial implications for the optimal application of SABR to early stage lung cancer. EF5-PET imaging could be useful as a risk stratification factor for clinical trials of lung cancer SABR, and could ultimately be used to individualize therapy for patients with early stage lung cancer.

REFERENCES:

None to date.

APPENDICES:

None.